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Hrs1p/Mcp6p on the Meiotic SPB Organizes Astral Microtubule Arrays for Oscillatory Nuclear Movement

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Summary

Microtubules and the motor protein dynein play pivotal roles in the movement and positioning of the nucleus and cytoplasmic organelles in a cell. In fission yeast, oscillatory movement of the nucleus termed horsetail nuclear movement (HNM) has been observed during meiotic prophase [1, 2]. HNM is led by an astral microtubule array emanating from the spindle pole body (SPB), a centrosome-equivalent organelle in yeasts, aided by the dynein-dynactin complex, and is proposed to facilitate the alignment of homologous chromosomes necessary for efficient meiotic recombination [3–8]. Here we show that a meiosis-specific SPB component Hrs1p (also known as Mcp6p [9]) is a key molecule to remodel microtubules into the horsetail-astral array (HAA). Deletion of Hrs1p impaired HAA formation, leading to compromised HNM. Ectopic expression of Hrs1p during the mitotic cell cycle resulted in the formation of a HAA-like astral microtubule array, which drove an oscillatory nuclear movement in interphase cells. Hrs1p interacted with components of the γ-tubulin ring complex (γ-TuRC) as well as with a meiotic SPB component. We propose that Hrs1p facilitates formation of the HAA, responsible for the vigorous HNM, by stabilizing connection between the SPB and minus ends of microtubules.

Results and Discussion

Hrs1p Localizes to the Meiotic SPB during Meiotic Prophase and Is Required for the Vigorous HNM

It has yet to be elucidated how cells switch from the vegetative microtubule array to the HAA in fission yeast. We cloned the *hrs1* gene (which was initially discovered during a search for novel SPB components involved in sexual differentiation, and is required for horse-tail movement, see below), which encoded a protein with coiled-coil motifs, a common feature of SPB core components. This gene is transcriptionally upregulated during sexual differentiation [9, 10]. The localization of Hrs1p was examined by tagging *hrs1* with green fluorescent protein (GFP). Hrs1p-GFP, which was apparently functional (see Figure S1D in the Supplemental Data available with this article online), appeared at the SPB upon conjugation of haploid cells, persisted until the onset of meiosis I, and disappeared thereafter (Figure 1A, Figure S1A). Similar results have been obtained in an independent study [9] in which the gene is termed *mcp6*. In this paper we call it *hrs1* throughout.

In addition to the localization to the SPB, several foci with a weak Hrs1p-GFP signal were often observed in zygotes undergoing horsetail nuclear movement (Figure 1A). No Hrs1p-GFP signal could be detected in mitotically growing cells. Hrs1p seemed to emerge in response to the pheromone signal, because it was observed in *mam2* mutant cells that developed a mating projection responding to the pheromone but were arrested before conjugation (Figure S1B) [11, 12]. The Hrs1p-GFP signal was also evident in azygotic diploid cells undergoing HNM (Figure 1B), and its temporal localization was confirmed by analysis of diploid cells undergoing meiosis synchronously (Figure S1C).

This horsetail-period-specific localization of Hrs1p indicated its possible function in HNM. The *hrs1* gene was dispensable for mitotic growth, but HNM was severely impaired in *hrs1Δ* during both zygotic and azygotic meiosis (Figure S1E and data not shown). Two phenotypes that are commonly seen among mutants deficient in HNM, namely aberrant spore formation and reduced meiotic recombination [4, 5, 8, 13], were also noticed in zygotic *hrs1Δ* (Figure S1D and data not shown). These results were consistent with the report by others that Hrs1p(Mcp6p) is required for HNM and meiotic recombination [9]. Interestingly, however, azygotic asci arising from diploid *hrs1Δ* cells did not show an apparent defect in spore formation (Figure S1D), suggesting that Hrs1p function might be related to a process specific to zygotes, such as karyogamy (see below). Crosses between *hrs1Δ* and wild-type cells yielded zygotes with no apparent deficiency in HNM and sporulation, indicating that Hrs1p is sufficient if supplied from one parent (Movies S1 and S2 and Figure S1D).

Hrs1p Is Required for the Maintenance of the HAA

It has been reported that HNM is dependent on dynein and the microtubule cytoskeleton [3, 4]. The dynein heavy chain in fission yeast, Dhc1p, is expressed exclusively during meiosis and localizes along microtubules, at their plus ends, and at the SPB [4, 14]. In *hrs1Δ* cells, the distribution of GFP-Dhc1p was apparently unaffected: it was still seen along microtubules and at a few foci (Figure 1C, Movies S3 and S4). The intensity of the GFP-Dhc1p signal in these cells was comparable to that in the wild-type. It therefore seemed unlikely that the impaired HNM in *hrs1Δ* cells arose from mislocalization of Dhc1p.

Closer observation of the microtubule structure using fixed *hrs1Δ* cells, which allowed detailed 3D analysis using a scanning confocal microscope, revealed that they lacked HAA bundles accompanied by a focused microtubule-organizing center (MTOC) (Figure 1D; Movies S5 and S6). Instead, nucleation of microtubules was more random and they were dispersed, suggesting that Hrs1p was required for HAA formation led by a unique

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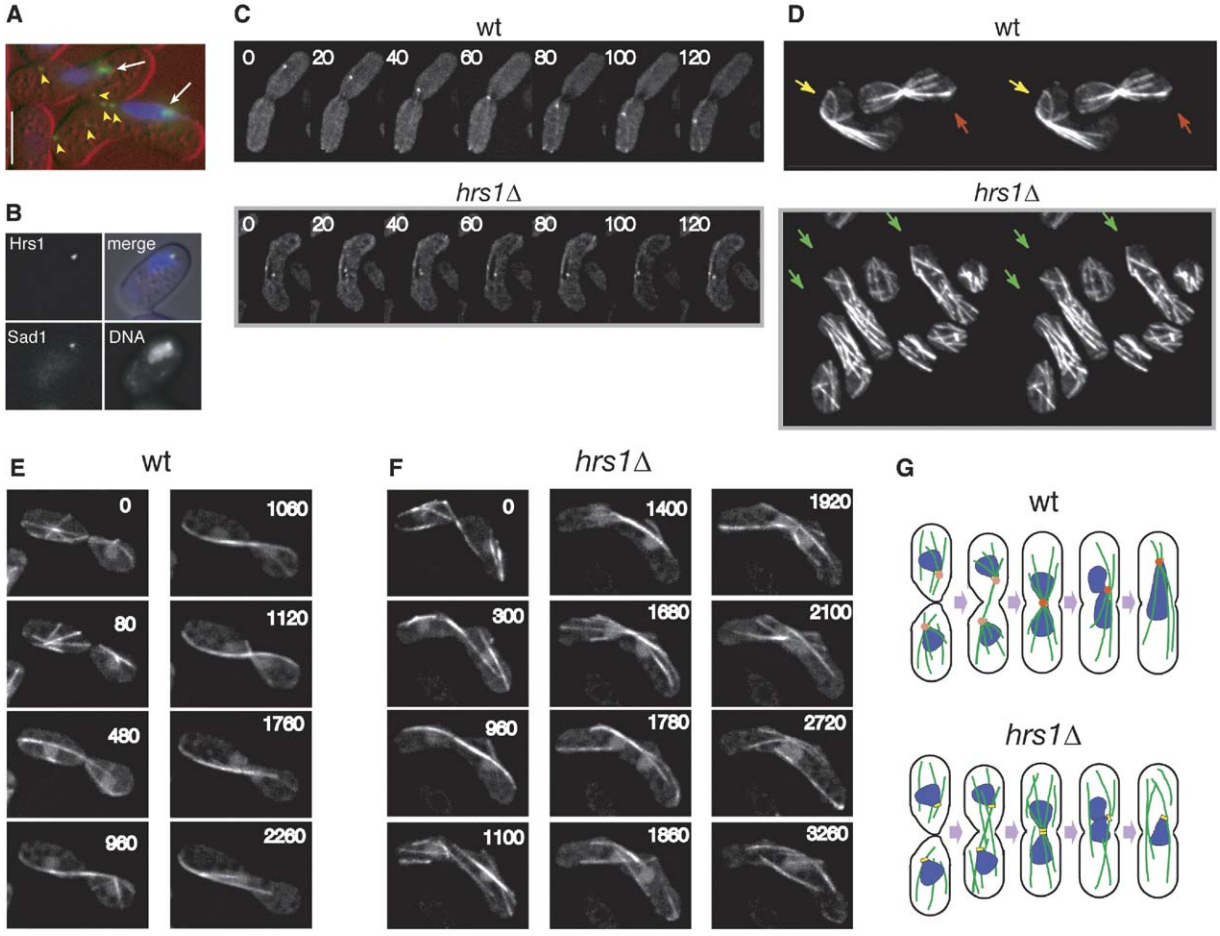

Figure 1. Hrs1p Localizes to the SPB and Is Required for the Stable HAA Formation

(A) The *h*^{90} *hrs1-GFP* strain harboring GST-NLS-CFP to visualize the nuclei was subjected to sexual differentiation and a live cell image was captured. Ten slices of images were taken at 0.5 μm intervals along the z axis and the maximum projection of Z series is shown. White arrows indicate strong Hrs1p-GFP signals at the SPBs, and weak Hrs1p-GFP foci are indicated by yellow arrowheads. Scale bar equals 5 μm.

(B) Hrs1p localizes to the SPB in azygotic diploid cells. Homothalic diploid cells carrying Hrs1p-GFP and Sad1p-DsRed were subjected to sexual differentiation, and a live cell image of a single focal plane was taken. Hrs1p-GFP and Sad1p-DsRed colocalized in the image. DNA was visualized by Hoechst 33342.

(C) Localization of Dhc1p is unaffected in the *hrs1Δ* mutant. *h*^{90} *Pnmt1-GFP-dhc1<<kan*^{r} cells (boxed in black) and *h*^{90} *Pnmt1-GFP-dhc1<<kan*^{r} *hrs1::ura4*$^{+}$ cells (boxed in gray) were cultured in EMM2 without thiamine for 16 hr to induce GFP-Dhc1p and then subjected to sexual differentiation. Live images of GFP-Dhc1p during the horsetail period were taken in multiple focal planes with a microscope equipped with Nipkow confocal unit at 20 s intervals. Images of maximum projection are shown. Numbers indicate time (in seconds) from the start of filming.

(D) Stereographic confocal images of the microtubule structure in *h*^{90} wild-type cells (boxed in black) and *h*^{90} *hrs1Δ* cells (boxed in gray). A wild-type zygote undergoing karyogamy is indicated by the red arrow and one in meiotic prophase by the yellow arrow. Three zygotes of the *hrs1Δ* mutant corresponding to meiotic prophase are indicated by green arrows.

(E and F) *h*^{90} wild-type cells (E) or *h*^{90} *hrs1Δ* cells (F) were subjected to sexual differentiation, and the behavior of microtubules at karyogamy and the following horsetail period was recorded every 20 s in multiple focal planes using a microscope equipped with a Nipkow confocal unit. Projections of representative frames are shown. Numbers indicate time (in seconds) from the start of filming.

(G) A schematic illustration of microtubule structure in wild-type and *hrs1Δ* cells. The SPB, microtubules, and nuclei are shown in yellow, green, and blue, respectively. Hrs1p is depicted as an orange dot. In the *hrs1Δ* mutant, stable HAA architecture is missing.

MTOC. Sad1p, an SPB component, stayed as a single focus in *hrs1Δ* cells, suggesting that SPB integrity was unaffected by the loss of Hrs1p function (Figure S2).

To understand the mechanism of HAA formation, we performed live observation of wild-type and *hrs1Δ* cells expressing a GFP-α-tubulin fusion protein (Movies S7 and S8; selected prints are given in Figures 1E and 1F). Our observations on wild-type cells (Movie S7) were consistent with a previous report [3]: an astral microtubule array radiating from the SPB was produced before cytoplasmic fusion in each mating partner (Figure 1E, time 0, 80) [15]. Upon conjugation, a solid microtubule bundle ran through the zygote (time 480), which might be nucleated from additional MTOCs formed at the projection tips [15]. The MTOCs on each nucleus tracked along the microtubule bundle until they met (time 960–

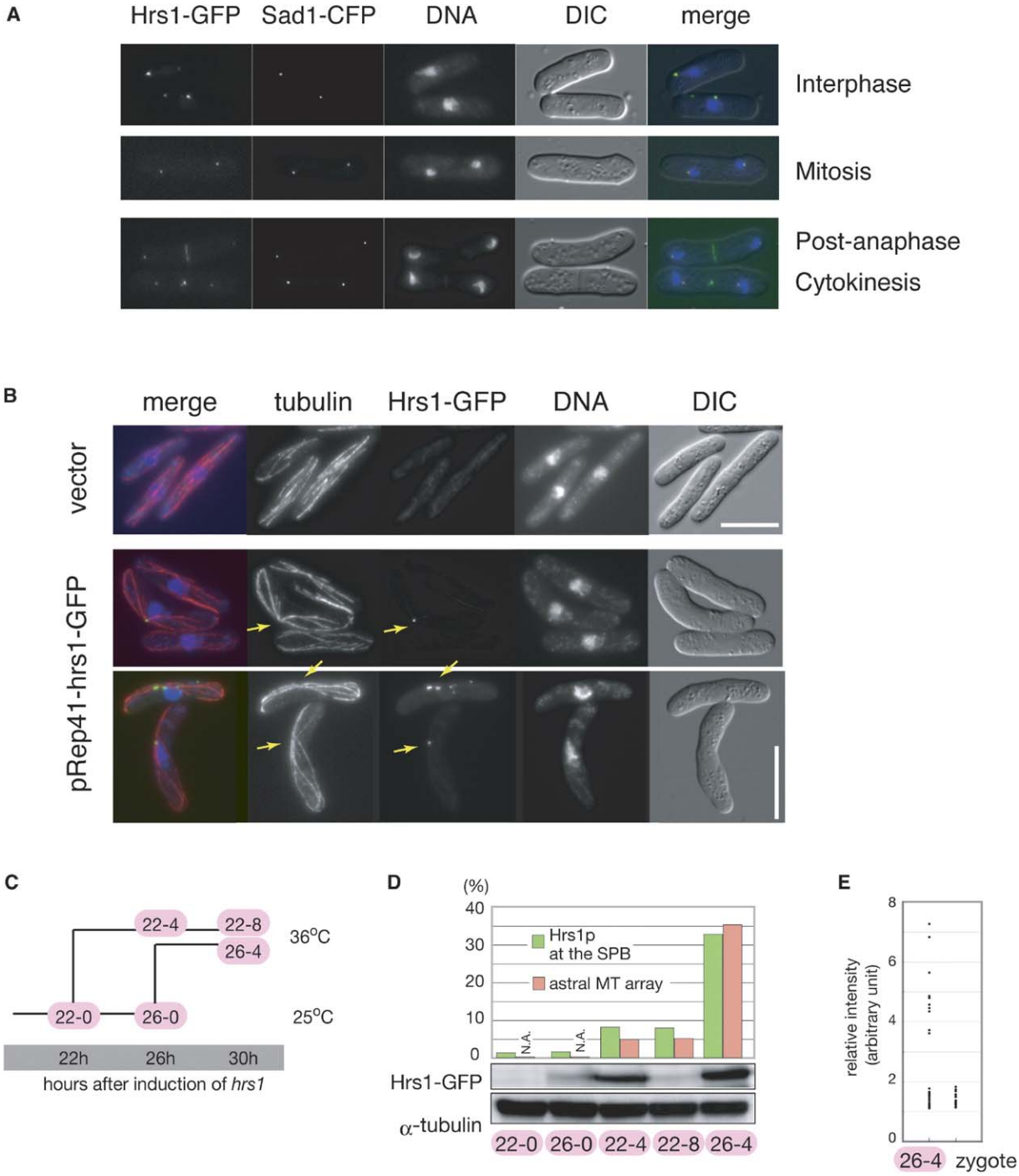

Figure 2. Hrs1p Mediates the HAA Formation

(A) Ectopically expressed Hrs1p during the mitotic cell cycle localized to the SPB and MTOC. Live images of cells expressing Hrs1p-GFP and Sad1p-CFP were taken. Eight slices of images were taken at 0.5 μm intervals along the z axis and the maximum projection of Z series is shown. DNA was visualized by Hoechst 33342. The host cell harbored the *cdc25.22* mutation and was slightly elongated even at the permissive temperature.

(B) Ectopic expression of Hrs1p induced the HAA-like structure in G2-arrested cells. Expression of *hrs1-GFP* was induced from the *nmt41* promoter by depleting thiamine for 24 hr in *cdc25.22* cells at the permissive temperature. Cells were then shifted to 36°C and incubated for 220 min to arrest cells in G2 phase. Cells were then fixed and processed for staining of tubulin and Hrs1p-GFP. Yellow arrows indicate cells with a Hrs1p-GFP signal. Scale bars equal 15 μm.

(C) A schematic representation of the experimental design. h^- *cdc25.22* cells carrying pRep41-*hrs1-GFP* were incubated for either 22 or 26 hr in the absence of thiamine at the permissive temperature (25°C) to induce Hrs1p-GFP expression. The culture was then shifted to the nonpermissive temperature (36°C) to arrest cells at the *cdc25*-execution point (late G2). Five types of samples, denoted 22-0, 26-0, 22-4, 22-8, and 26-4, were prepared, in which the former number indicates the incubation time (hours) at 25°C and the latter that at 36°C.

1120) and eventually generated an HAA. Two nuclei ran after each other for a while until karyogamy took place, which was followed by HNM (Movie S7).

In *hrs1Δ* cells (Movie S8), formation of an astral microtubule array from an MTOC on each nucleus was not obvious before conjugation (Figure 1F, time 0, note that cell conjugation had already taken place). Whereas a solid microtubule bundle running through the zygote was formed as in wild-type cells (time 300), MTOCs arranging microtubule arrays were scattered and it took longer for the nuclei to come together (time 0–1920). The equivalent timing for the wild-type zygote shown in Figure 1E could be time 480–1120. Some *hrs1Δ* zygotes failed to perform karyogamy, explaining the haploid-specific sporulation-deficient phenotype (Figure S1D). Occasionally, very unstable HAA-like structures with a single MTOC were observed (for example, Figure 1F, time 1680). This indicated that Hrs1p might not be required for the HAA formation per se but might function in stabilizing the HAA, as depicted in Figure 1G.

Ectopic Expression of Hrs1p Induces an HAA-like Microtubule Structure

We explored whether the presence of Hrs1p could induce an HAA-like structure in mitotically growing cells. Since Hrs1p is not normally expressed in these cells, Hrs1p-GFP expression was driven by a plasmid-borne thiamine-repressible *nmt41* promoter. Culturing the cells in the absence of thiamine for 32 hr at 25°C affected neither the doubling time nor the mitotic index (data not shown). After induction, Hrs1p-GFP was localized not only to the SPB but also to the equatorial MTOC (eMTOC), which is responsible for organizing the astral microtubule structure termed post anaphase array (PAA) [16–21] (Figure 2A). This suggested that Hrs1p had an intrinsic affinity for either MTOCs or the minus ends of microtubules or both. Cells overexpressing Hrs1p-GFP during vegetative growth were frequently bent and their nuclei were often drifting away from the cell equator (Figure 2A, interphase).

To observe the effect of Hrs1p overexpression on microtubule architecture, we employed G2-arrested *cdc25.22* cells, because they lacked the astral microtubule structure derived from the PAA. In the majority of these cells, microtubule arrays were organized from multiple interphase MTOCs (iMTOCs) [22] and less than 1% of the cells had a single MTOC. After either 22 hr or 26 hr induction of Hrs1p at the permissive temperature, cells were shifted to the restrictive temperature and incubated for 4 hr (Figure 2C). They were fixed and mictotubules were visualized by indirect immunofluorescent staining (Figure 2B). Approximately 5% of the cells subjected to the induction for 22 hr (Sample 22-4) contained a single MTOC that nucleated multiple microtubule bundles to form a pseudo HAA (p-HAA) structure (Figure 2D). The population of cells with a p-HAA increased to 35% if the induction was extended to 26 hr (Sample 26-4), indicating that an increase of the amount of Hrs1p before the arrest drove formation of a p-HAA. The Hrs1p-GFP signal was detected at the MTOC for the p-HAA (Figure 2B, yellow arrows), which coincided with the SPB (data not shown). Two-thirds of the cells carrying Hrs1p-GFP at the SPB emitted green fluorescence of comparable intensity to meiotic cells undergoing prophase I (Figure 2E). The rest of the cells showed fluorescence up to several times stronger or multiple Hrs1p-GFP dots (Figure 2E), probably because they carried more copies of the *hrs1*-GFP plasmid than others. Altogether, these results suggested that ectopic expression of a physiological level of Hrs1p could induce the p-HAA.

We noticed that extended incubation of *cdc25.22* cells under the restrictive temperature following the 22 hr induction (Sample 22-8) did not enhance p-HAA formation, and that the amount of Hrs1p was greatly reduced in these cells (Figure 2D). This indicated that the stability of Hrs1p might be regulated in a cell cycle-dependent manner and a p-HAA might be generated in early G2 phase before arresting at the *cdc25* execution point.

Ectopic Expression of Hrs1p during Mitosis Induced an Oscillatory Nuclear Movement Driven by Astral Microtubule Arrays

We observed frequent nuclear movement in cells expressing Hrs1p during mitotic growth (Movie S10). However, we recognized also that wild-type cells showed oscillatory nuclear movement shortly after the cytokinesis (early G2 cells) (Movie S9). To assess these movements quantitatively, we employed a heterothallic haploid strain harboring Sid4p-GFP [23] and a GFP-α-tubulin fusion protein, which visualized the SPB and microtubules simultaneously. Six cells of this strain longer than 9 μm (mid-late G2 cells) were analyzed as controls (Figure 3B). All the cells had an interphase microtubule structure organized from multiple iMTOCs and no astral microtubule array (Figure 3E, Movie S11). The movement of the SPB was moderate and its location was restricted to the center of the cells (Figure 3B). In contrast, early G2 cells shorter than 9 μm showed a more dynamic oscillatory movement of the SPB, as mentioned above (Figures S3A and S3B and Movie S11).

When Hrs1p was ectopically expressed in the above strain, it induced the p-HAA structure in roughly 25% of the cells. Six cells longer than 9 μm that had the p-HAA were chosen to trace the position of the SPB

(D) The proportion of cells with a Hrs1p-GFP signal at the SPB (for all samples) and that of cells carrying an astral MT array (for 22-4, 22-8, and 26-4) were scored. 200 cells were counted for each sample. The amount of Hrs1p-GFP in each sample and that of α-tubulin as a control were measured by immunoblotting of total cell extracts.

(E) Comparison of the intensity of the Hrs1p-GFP signal on the SPB in sample 26-4 and that in wild-type zygotes undergoing meiosis. The data were taken from 31 cells of sample 26-4 and 19 zygotes of h^{90} *hrs1-GFP* cells. The images were captured and processed under the same conditions, and the intensities of the fluorescence were measured using ImageJ software (Wayne Rasband, National Institute for Mental Health). The zygotes showed a distribution of intensities between 1 and 2 arbitrary units, and two-thirds of the cells in sample 26-4 fell into this range.

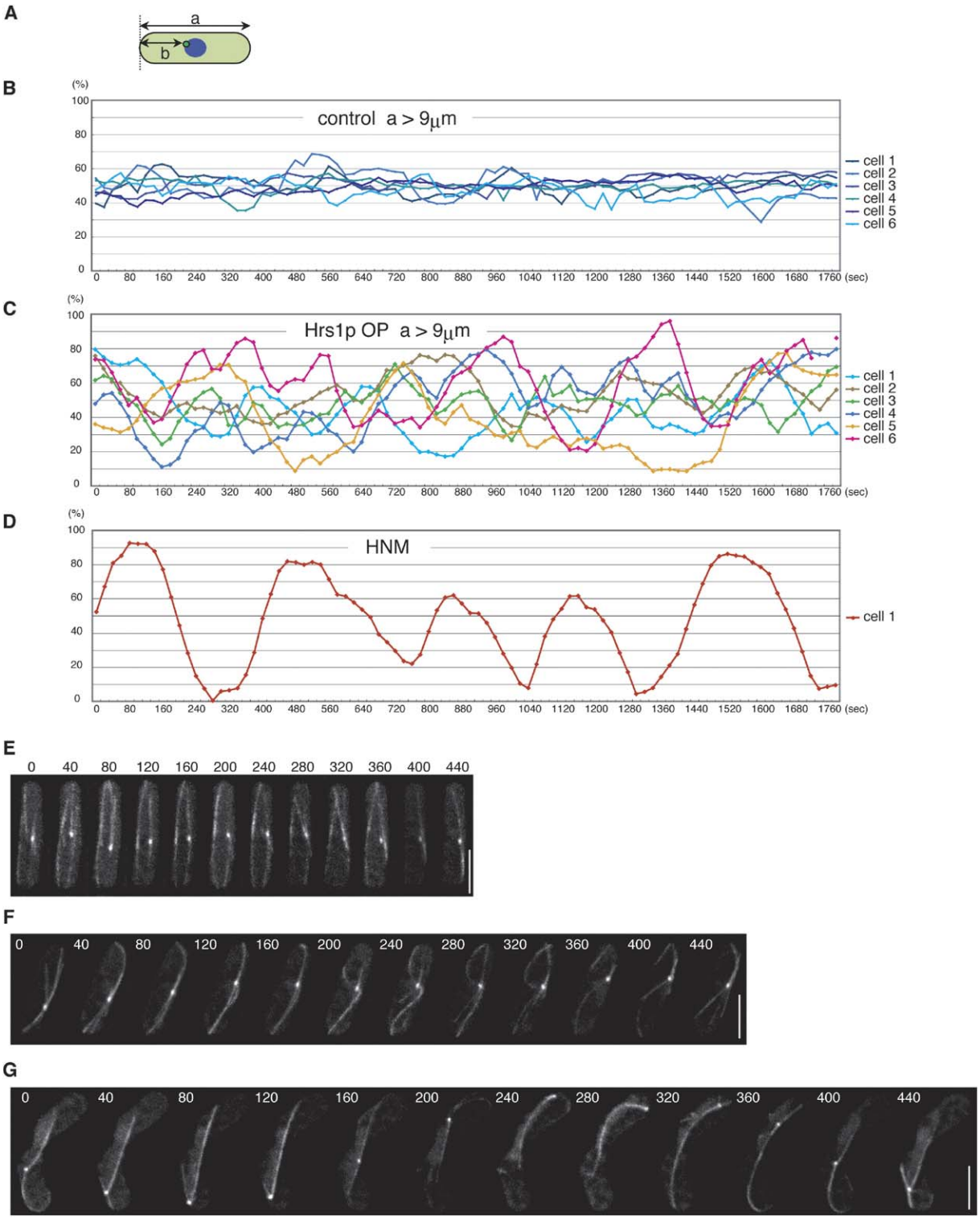

Figure 3. Ectopic Expression of Hrs1p during the Mitotic Cell Cycle Induces Oscillatory Nuclear Movement Driven by HAA-like Microtubule Structure

(A) An illustration to schematize the measurements. "a" represents the length of a cell, whereas "b" represents the distance from a cell end to the SPB.

(B and C) Relative location of the SPB (b/a) in h^- wild-type cells expressing Sid4p-GFP (an SPB marker) and GFP-tubulin, transformed either with a control vector (B) or pRep2-*hrs1-HA* (C) and incubated for 20 hr at 30°C in the absence of thiamine to induce Hrs1p-HA and GFP-tubulin. 3D live images (8 focal planes, 0.65 μm interval) were taken every 20 s using a microscope equipped with a Nipkow confocal unit. Measurements were done based on the projection images of each time point. Cells longer than 9 μm were chosen.

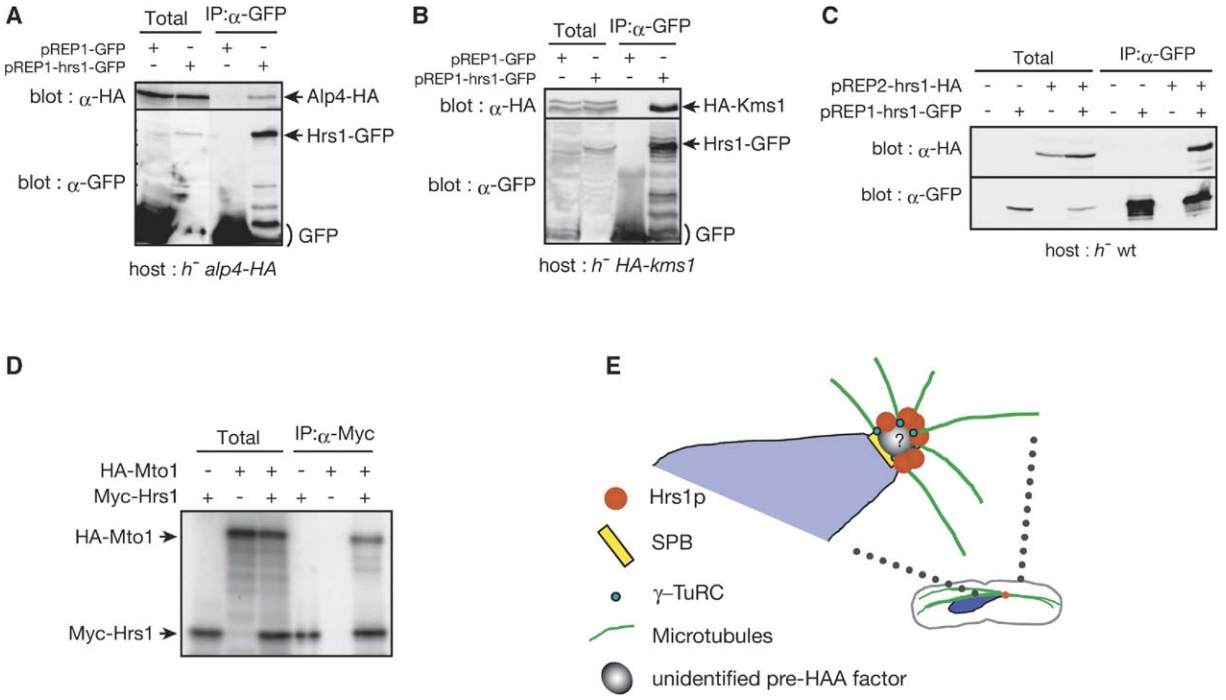

Figure 4. Hrs1p Interacts with a Component of γ-TuRC, an SPB Component, Hrs1p Itself, and a γ-TuRC-Associated Protein

(A) Hrs1p-GFP ectopically expressed in *h⁻ alp4⁺-3HA* during the mitotic cell cycle coimmunoprecipitated Alp4p-HA, while control GFP did not. The amounts of Hrs1p-GFP (arrow) and GFP (bracket) in the immunocomplexes are shown in the lower panel. "Total" represents 10% of the cell extract subjected to immunoprecipitation.

(B) Hrs1p-GFP ectopically expressed in *h⁻ nmt1-3HA-kms1* during the mitotic cell cycle coimmunoprecipitated 3HA-Kms1p, while control GFP did not. Multiple bands of the 3HA-Kms1p in the total cell extracts represent different phosphorylation states (M. Shimanuki and O. Niwa, personal communication). The amounts of Hrs1p-GFP (arrow) and GFP (bracket) in the immunocomplexes are shown in the lower panel. "Total" represents 10% of the cell extract subjected to immunoprecipitation.

(C) Hrs1p-GFP and Hrs1p-HA were simultaneously expressed during the mitotic cell cycle and immunoprecipitation was performed with anti-GFP antibodies. Hrs1p-GFP coimmunoprecipitated Hrs1p-HA, indicating that Hrs1p may form a dimeric or oligomeric complex. "Total" represents 10% of the cell extract subjected to immunoprecipitation.

(D) Hrs1p directly interacts with Mto1p. In vitro translated Myc-Hrs1p and HA-Mto1p were produced in the presence of ^{35}S-Methionine and immunoprecipitation assays were performed. "Total" represents 50% of the reaction mixture subjected to immunoprecipitation assays.

(E) Schematic illustration of our working hypothesis. Minus ends of microtubules decorated with γ-TuRC are brought to the SPB to form an unstable pre-HAA structure. The Hrs1p complex stabilizes it by interacting with γ-TuRC and the SPB. Minus ends of some microtubules may interact directly with the SPB as they do in the mitotic cell cycle.

(Figure 3C). The SPB often wandered away from the center of the cell, performing an oscillatory movement led by the p-HAA (Figure 3F, Movie S12). This result indicates that remodeling of microtubules into an astral array by Hrs1p may play a crucial role in the regulation of nuclear movement.

It has been proposed that the SPB is subject to a strict positional control during interphase and is responsible for nuclear positioning [18]. A balance of pushing forces by interphase antiparallel microtubules, emanating from multiple iMTOCs located around the nucleus, may also contribute to nuclear positioning [22]. Thus, ectopic expression of Hrs1p may have disturbed the positional regulation of the SPB and/or the balance of the pushing forces, resulting in the drift of nuclei away from the cell equator.

Compared to HNM (Movie S13), the speed and amplitude of the mitotic nuclear motion induced by Hrs1p was less drastic and the periodicity of the oscillation pattern was not strictly regulated (compare Figures 3C and 3F with 3D and 3G). This may be because of a lack of motor protein Dhc1p [4, 14], meiosis-specific dynactin component Ssm4p [8], and other proteins indispensable for HNM.

Mutants defective in microtubule regulation tend to show problems in positioning the nuclei in the center of the cell [20, 24–27]. To examine if any perturbation of MT organization could induce the HNM-like oscillatory

(D) The b/a value calculated for typical live images of HNM, taken and processed in the same way as above.

(E) Representative projected images of a control mitotic cell in mid-late G2, analyzed in (B).

(F) Representative projected images of a mid-late G2 cell ectopically expressing Hrs1p, analyzed in (C).

(G) Representative projected images of a zygote undergoing HNM, analyzed in (D). In (E), (F), and (G), the numbers indicate time in seconds and the images are presented in the same magnification. Scale bar equals 5 μm.

nuclear movement, we traced the position of the SPB in the mutant defective in *mto1/mod20/mbo1* (*mto1* hereafter). Microtubule dynamics in *mto1Δ* showed pleiotropic problems, and the position of the SPB was often away from the cell center, as has been reported [25–27]. Although nuclear movement was observed in the *mto1* mutant cells, it was random and not oscillatory as seen for Hrs1p overproduction (Movies S14 and S15). Therefore, the HNM-like nuclear movement caused by ectopic expression of Hrs1p is apparently a specific effect and is unlikely to be a common outcome of disturbed MT dynamics.

Hrs1p Interacts with γ-TuRC and a Meiotic SPB Component

To gain insights into the mechanism of HAA formation mediated by Hrs1p, we examined the interaction of Hrs1p with Kms1p, Alp4p, and Mto1p. Kms1p is an SPB component required for the integrity of the meiotic SPB [5, 28]. HNM is severely impaired in the absence of Kms1p. Alp4p is a component of the γ-TuRC, which resides at the minus ends of microtubules [20]. Mto1p is a γ-TuRC-associated protein [25, 26]. In yeast two-hybrid assays, Hrs1p interacted with the Kms1p N-terminal half, Alp4p, and Hrs1p itself (Figure S3A). These interactions were further confirmed by immunoprecipitation (Figures 4A–4C). Although Hrs1p failed to interact with Mto1p in the two-hybrid assays (data not shown), in vitro translated Hrs1p and Mto1p showed a specific interaction (Figure 4D). Thus, Hrs1p, Kms1p, Alp4p, and Mto1p are likely to form a complex on the SPB.

We next investigated whether Hrs1p function could affect the localization of Alp4p. During meiotic prophase, Alp4p localizes to the SPB (Figure S3B, wt). In *hrs1Δ*, the concentration of Alp4p to the SPB was lost and the signal was dispersed around the nucleus (Figure S3B, *hrs1Δ*). This dispersion was specific to zygotes undergoing meiotic prophase, and upon the onset of meiosis I, localization of Alp4p to the SPB was restored in *hrs1Δ* (data not shown). In growing cells, Alp4p is reported to reside on scattered "satellites" around the nucleus at interphase in addition to the SPB [29]. These satellites represent the iMTOCs from which interphase cytoplasmic microtubules are nucleated. We examined the effect of ectopic Hrs1p expression using cells treated with hydroxyurea (HU). After the HU treatment for 4 hr at 30°C, more than 90% of the cells were arrested at the G1-S boundary, having neither a spindle nor a PAA but carrying Alp4p patches at satellites (91.3%, n = 218) and a concentration of it at the SPB (Figure S3C, control). When Hrs1p was expressed in these cells, signals at satellites were precipitated into a single Alp4p signal at the SPB in many of them (53.6%, n = 207) (Figure S3C, pREP1-*hrs1*).

Based on these observations, we propose a model depicted in Figure 4E. Once the pre-HAA structure is organized, Hrs1p acts to stabilize it by attracting the minus ends of microtubules via γ-TuRC and fixes them to the SPB. It has been shown that assembly and disassembly of microtubules during HNM takes place mainly at their plus ends distal to the SPB and that the minus ends proximal to the SPB are relatively static [14]. This observation fits well with our model, which does not postulate a special MTOC activity at the SPB to nucleate multiple microtubule bundles for HAA organization.

One of the distinctive features of microtubules in the HAA is that their plus ends tend to curl around cell ends (Figures 1E and 3G) [3, 15]. We noticed a similar trend in microtubules organized into the p-HAA by ectopic expression of Hrs1p in mitotic cells (Figures 2B and 3F). This suggests that Hrs1p may also affect the kinetics of polymerization and depolymerization of microtubules at the plus ends. The Hrs1p foci of weak intensity observed in Figure 1A may imply its possible involvement in the regulation of microtubule dynamics. Alternatively, fixation of the minus ends to the SPB or the resultant HAA arrangement may affect the dynamics of microtubules at plus ends.

Currently, we suppose that the formation of a pre-HAA involves yet another key factor than Hrs1p, because *hrs1Δ* cells could still generate pre-HAA architecture, although it was very unstable (Figure 1F). For example, a putative "pre-HAA factor" as depicted in Figure 4E may physically arrange the pre-HAA architecture, which is subsequently stabilized by Hrs1p. Alternatively, certain regulation/modification of the components of γ-TuRC or the SPB may be essential for the arrangement. As a pre-HAA-like structure can be formed in the *kms1* mutant [5], we speculate that Kms1p is unlikely to be the pre-HAA factor itself. Rather, Mto1p appears to be a good candidate for this factor for a couple of reasons. First, its role in generating the PAA during the mitotic cell cycle has been established [25, 26]. Second, Hrs1p physically interacts with Mto1p. Third, HAA formation during meiotic prophase was severely impaired in the absence of Mto1p function (our unpublished observation). Obviously, however, further analysis of the meiotic SPB is needed to fully understand the formation of HAA microtubule bundles and the timely induction of HNM.

Supplemental Data

Supplemental Data include four figures, one table, 15 movies, and Supplemental Experimental Procedures and can be found with this article online at http://www.current-biology.com/cgi/content/full/15/16/1479/DC1/.

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